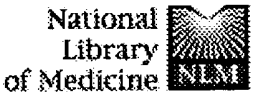

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Search	Most Recent Queries	Time	Result
#3	Search **(BVDV or bovine viral diarrhea virus) AND (HCV or hepatitis C)** Field: **Title/Abstract,** Limits: **Publication Date to 1999/06/04**	12:42:33	75
#4	Search **#3 AND chimer*** Field: **Title/Abstract,** Limits: **Publication Date to 1999/06/04**	12:37:29	1
#2	Search **(BVDV or bovine viral diarrhea virus) AND (HCV or hepatitis C)** Field: **Title/Abstract**	12:35:27	125
#1	Search **(BVDV or bovine viral diarrhea virus) AND (HCV or hepatitis C)**	12:35:21	130

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		DB=PGPB,USPT,EPAB,JPAB,DWPI; PLUR=YES; OP=OR	
☐	L6	l1 not l5	41
☐	L5	L4 and l3	26
☐	L4	(BVDV or bovine adj viral adj diarrhea adj vir$3) same (hepatitis adj C or HCV)	221
☐	L3	L1 and (hepatitis adj C)	34
☐	L1	(BVDV or bovine adj viral adj diarrhea adj vir$3) same (vector or heterologous or chimer$ or exogenous or fusion)	67

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First Hit

L5: Entry 14 of 26 File: PGPB Mar 21, 2002

DOCUMENT-IDENTIFIER: US 20020034732 A1
TITLE: COMPOSITIONS AND METHODS FOR DETERMINING ANTI-VIRAL DRUG SUSCEPTIBILITY AND RESISTANCE AND ANTI-VIRAL DRUG SCREENING

Summary of Invention Paragraph:
[0005] The small and efficient genomes of viruses have lent themselves to the intensive investigation of the molecular genetics, structure and replicative cycles of most important human viral pathogens. As a consequence, the sites and mechanisms have been characterized for both the activity of and resistance to anti-viral drugs more precisely than have those for any other class of drugs. (Richman (1994) Trends Microbiol. 2, 401-407). The likelihood that resistant mutants will emerge is a function of at least four factors: 1) the viral mutation frequency; 2) the intrinsic mutability of the viral target site with respect to a specific anti-viral; 3) the selective pressure of the anti-viral drug; and, 4) the magnitude and rate of virus replication. with regard to the first factor, for single stranded RNA viruses, whose genome replication lacks a proofreading mechanism, the mutation frequencies are approximately 3.times.10.sup.-5 per base-pair per replicative cycle (Holland et al. (1992) Curr. Topics Microbiol Immunol. 176, 1-20; Mansky et al. (1995) J Virol. 69, 5087-94; Coffin (1995) Science 267, 483-489). Thus, a single 10 kilobase genome, like that of human immunodeficiency virus (HIV) or hepatitis C virus (HCV), would be expected to contain on average one mutation for every three progeny viral genomes. As to the second factor, the intrinsic mutability of the viral target site in response to a specific anti-viral agent can significantly affect the likelihood of resistant mutants. For example, zidovudine (AZT) selects for mutations in the reverse transcriptase of HIV more readily in vitro and in vivo than does the other approved thymidine analog d4T (stavudine).

Summary of Invention Paragraph:
[0009] Hepatitis C Virus (HCV)

Summary of Invention Paragraph:
[0010] Hepatitis C virus (HCV) infection occurs throughout the world and, prior to its identification, represented the major cause of transfusion-associated hepatitis. The seroprevalence of anti-HCV in blood donors from around the world has been shown to vary between 0.02% and 1.23%. HCV is also a common cause of hepatitis in individuals exposed to blood products. There have been an estimated 150,000 new cases of HCV infection each year in the United States alone during the past decade (Alter 1993, Infect. Agents Dis. 2, 155-166; Houghton 1996, in Fields Virology, 3rd Edition, pp. 1035-1058).

Summary of Invention Paragraph:
[0011] The hepatitis C virus (HCV) is a member of the flaviviridae family of viruses, which are positive stranded, non-segmented, RNA viruses with a lipid envelope. Other members of the family are the pestiviruses (e.g. bovine viral diarrheal virus, or BVDV, and classical swine fever virus, or CSFV), and flaviviruses (e.g. yellow fever virus and Dengue virus). See Rice, 1996 in Fields Virology, 3rd Edition, pp. 931-959. Molecular dissection of HCV replication and hence understanding the functions of its encoded proteins, while greatly advanced by the isolation of the virus and sequencing of the viral genome, has been hampered by the lack of an efficient cell culture system for production of native or recombinant HCV from molecular clones. However, low-level replication has been

observed in several cell lines infected with virus from HCV-infected humans or chimpanzees, or transfected with RNA derived from cDNA clones of HCV.

Brief Description of Drawings Paragraph:
[0066] FIG. 8: Resistance Test Vectors (BVDV NS3/4A Chimeras, luc Fusion Protein)

Brief Description of Drawings Paragraph:
[0067] A diagrammatic representation of the genome of BVDV is shown at the top. HCV protease cleavage sites are indicated by grey triangles, and BVDV protease cleavage sites are represented by crosshatched diamonds (signal peptidase and NS2/3 protease cleavage sites are not shown). The resistance test vector pXBVDV(HCVNS3)luc contains the BVDV structural protein genes, BVDV NS2, HCV NS3/4A protease, and BVDV NS4B and NS5; the cleavage sites in the nonstructural protein region are altered so that they are recognized by the HCV NS3/4A protease. The luciferase reporter gene is expressed as a fusion with the chimeric polyprotein, and released by cleavage by HCV NS3/4A.

Brief Description of Drawings Paragraph:
[0068] FIG. 9: Resistance Test Vectors (BVDV NS5B Chimeras, luc Fusion Protein).

Brief Description of Drawings Paragraph:
[0069] The resistance test vector pXBVDV(HCVNS5B)luc comprising the BVDV structural protein genes, BVDV NS2, NS3/4A protease, NS4B and NS5A, and HCV NS5B; the cis-acting regulatory elements recognized by the NS5B polymerase, located in the 3' UTR and 5' UTR and amino terminal region of the C ORF, are derived from HCV. The luciferase reporter gene is expressed as a fusion with the chimeric polyprotein, and released by cleavage by BVDV NS3/4A.

Detail Description Paragraph:
[0165] Initiation of translation of the HCV polyprotein occurs via a cap-independent internal initiation mechanism. The 5' end of the viral RNA, comprising the untranslated region (UTR) and the first 369 nucleotides of the C open reading frame, contains a sequence and/or structure which directs cap-independent translation initiation (Tsukiyama-Kohara et al, J Virol. 66:1476, 1992; Wang et al, J Virol. 67:3338, 1993; Lu and Wimmer, PNAS 93:1412, 1996). Other viruses such as poliovirus (PV) (Pelletier and Sonenberg (1988), Nature, 334, 320-325), encephalomyocarditis virus (EMCV) (Jang et al. (1989), J. Virol. 63, 1651-1660), rhinovirus (RV) (Rohll et al. (1994), J. Virol. 68, 4384-4391), hepatitis A virus (HAV) (Brown et al. (1994), J. Virol. 68, 1066-1074; Glass et al. (1993) Virol. 193, 842-852), as well as the pestivirus, bovine viral diarrhea virus (BVDV) (Poole et al. (1995) Virology, 189, 285-292) to which HCV is closely related, employ similar mechanisms for translation initiation, altough the sequences which serve as the internal ribosome entry site (IRES) are different for each virus. Some cellular mRNAs are also known to initiate translation internally via an IRES (Macejak and Sarnow (1991), Nature, 353, 90-94). These RNA elements have been shown to be capable of directing translation initiation when located in between two open reading frames, as well as at the 5' end of RNAs. These bicistronic RNAs can be used to obtain expression of two proteins from the same RNA by independently directing the translation of both open reading frames.

Detail Description Paragraph:
[0199] Infectious recombinant virions are produced from cells transfected with two vectors: an IGVV containing an IG and the viral non-structural proteins, and a second vector, the packaging vector, containing the viral structural proteins (C/E1/E2; see FIG. 7). To generate infectious particles, the IGVV DNA (or its corresponding RNA, see Example 1, FIGS. 3B-3Alternatively, particles are pseudotyped with envelope glycoprotein genes from related flaviviruses such as BVDV or classical swine fever virus (CSFV). The pseudotyped viruses are used to establish of a cell culture system for single-cycle infection assays. Viruses produced in this manner can then be used to infect target cells, and luciferase

expression subsequently measured. This approach has the added advantage of minimizing the amount of manipulations performed with replication competent infectious agents.

Detail Description Paragraph:
[0207] HCV Protease Inhibitor Susceptibility and Resistance Test Using Resistance Test Vectors Comprising Patient-derived Segment(s) and a Functional Indicator Gene in an NS3/4A BVDV Chimeric Viral Vector.

Detail Description Paragraph:
[0209] A chimeric IGVV containing a functional indicator gene and the relevant portion(s) of HCV (for example, the NS3/4A protease domain) were designed with a backbone of a related virus which replicates well in culture. An example of such a virus is BVDV. A complete cDNA for the genome of BVDV has been assembled and shown to generate infectious RNA by in vitrotranscription (Vassilev et al. 1997, J. Virol. 71:471-478). The BVDV polyprotein is processed in a manner very similar to that of HCV, using both host (signal peptidase) and viral encoded proteases. The chimeric IGVV based on a BVDV backbone contains the NS3 protease domain or entire NS3/4A open reading frame of HCV which replaces the corresponding region of BVDV (FIG. 8). By mutating the cleavage sites normally recognized by BVDV NS3 protease to those recognized by HCV NS3/4A, replication of BVDV chimeric RNA and expression of the IG will be dependent on HCV NS3/4A activity.

Detail Description Paragraph:
[0210] Chimeric indicator gene viral vectors containing a functional indicator gene in an NS3/4A BVDV chimeric viral vector are constructed as follows. The IGVV contains the following elements in a 5' to 3' orientation: a promoter sequence, the BVDV 5' UTR, the C through NS2 regions of BVDV (NADL strain), the NS3/4A region of HCV, the HCV NS4A/4B cleavage site, the BVDV NS4B open reading frame, the HCV NS4B/SA cleavage site, the BVDV NS5A open reading frame, the HCV NS5A/5B cleavage site, the BVDV NS5B open reading frame, the luciferase open reading frame, the BVDV 3' UTR and a transcription terminator. In a second embodiment, the IGVV contains the luciferase open reading frame preceeded by an IRES in a similar configuration to that described in Example 2. In a third embodiment, the luciferase gene is expressed from a minigenome similar to that described in Examples 3 or 4.

Detail Description Paragraph:
[0213] Resistance test vectors containing a functional indicator gene in an NS3/4A BVDV chimeric viral vector are constructed from IGVVs described above and patient-derived HCV NS3/4A sequences as described in Example 1. The IGVV is modified to include PSAS for the insertion of NS3/4A-containing PDS (described in Example 1, see FIG. 3A).

Detail Description Paragraph:
[0218] HCV Drug Susceptibility and Resistance Test Using Resistance Test Vectors Comprising Patient-derived Segment(s) and a Functional Indicator Gene in an NS5B BVDV Chimeric Viral Vector.

Detail Description Paragraph:
[0220] A chimeric IGVV containing the BVDV structural and non-structural proteins, with the exception of NS5B which is derived from HCV, is designed with a backbone of BVDV. In addition, the BVDV 5' and 3' UTRs are replaced with the corresponding regions from HCV, to ensure recognition by the cognate polymerase (FIG. 9).

Detail Description Paragraph:
[0221] Indicator gene viral vectors containing a functional indicator gene in an NS5B BVDV chimeric viral vector are constructed as follows. The IGVV contains the following elements in a 5' to 3' orientation: a promoter sequence, the HCV 5' UTR, sequences from the N-terminus of the HCV C open reading frame required for IRES function, the Npro through NS5A regions of BVDV (NADL strain), the NS5B region of

HCV, the luciferase open reading frame, the HCV 3' UTR, and a transcription terminator. In a second embodiment, the IGVV contains the luciferase open reading frame preceeded by an IRES in a similar configuration to that described in Example 2. In a third embodiment, the luciferase gene is expressed from a minigenome similar to that described in Examples 3 or 4.

Detail Description Paragraph:
[0224] Resistance test vectors containing a functional indicator gene in an NS5B BVDV chimeric viral vector are constructed from IGVVs described above and patient-derived HCV NS5B sequences as described in Example 1. The IGVV is modified to include PSAS for the insertion of NS5B-containing PDS (described in Example 1, see FIG. 3A).

CLAIMS:

1. A method for determining susceptibility for an HCV anti-viral drug comprising: (a) introducing a resistance test vector comprising a patient-derived segment which comprises a hepatitis C virus gene and an indicator gene into a host cell; (b) culturing the host cell from (a); (c) measuring expression of the indicator gene in a target host cell; and (d) comparing the expression of the indicator gene from (c) with the expression of the indicator gene measured when steps (a)-(c) are carried out in the absence of the anti-viral drug, wherein a test concentration of the HCV anti-viral drug is present at steps (a)-(c); at steps (b)-(c); or at step (c).

37. A method for determining susceptibility for an HCV anti-viral drug comprising: (a) introducing a resistance test vector comprising a patient-derived segment which comprises a hepatitis C virus gene and a nonfunctional indicator gene into a host cell; (b) culturing the host cell from (a); (c) measuring expression of the indicator gene in a target host cell; and (d) comparing the expression of the indicator gene from (c) with the expression of the indicator gene measured when steps (a)-(c) are carried out in the absence of the HCV anti-viral drug, wherein a test concentration of the HCV anti-viral drug is present at steps (a)-(c); at steps (b)-(c); or at step (c).